

Wood decomposition by *Phellinus (Fomes) pini*: a scanning electron microscopy study¹

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Decomposition of white pine (*Pinus monticola* Dougl.) and western larch (*Larix occidentalis* Nutt.) by the white-pocket rot fungus, *Phellinus (Fomes) pini* (Thore ex Fr.) A. Ames, was investigated using scanning electron microscopy. White tissues surrounded by apparently sound wood characterized the decay. Latewood was first to be colonized and degraded. A selective degradation of lignin was observed. The middle lamella between tracheids was completely decomposed. The primary and secondary cell wall contained a macrofibrillar structure indicating lignin was removed leaving a cellulose framework. Macrofibrils 0.2–0.4 µm were present. In white-pocket regions, ray parenchyma was completely degraded. Sound wood, bordering white-pocket areas, usually consisted of early wood and resin-filled ray parenchyma. Fungal hyphae and bore holes were present in surrounding wood but decay typical of white-pocket rot was not observed. In addition to white-pocket rot, *P. pini* causes a brownish decay in wood near sporophores and punk knots that is ultrastructurally similar to decay by a white rot fungus. The potential use of white-pocket rot decay as a livestock feed and use of *P. pini* in a biological pulping process for the production of paper is discussed.

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La décomposition du pin argenté (*Pinus monticola* Dougl.) et du mélèze occidental (*Larix occidentalis* (Nutt.) par le champignon de la carie blanche alvéolaire, *Phellinus (Fomes) pini* (Thore ex Fr.) A. Ames, a été étudiée en microscopie électronique à balayage. La présence de tissus blanchâtres entourés de bois apparemment sain caractérise cette carie. Le bois tardif est le premier à être colonisé et dégradé. On observe une dégradation sélective de la lignine. La lamelle moyenne entre les trachéides est complètement décomposée. La paroi cellulaire primaire et secondaire contient une structure microfibrillaire, montrant que la lignine a été enlevée pour laisser une armature cellulosique. Il y a des macrofibrilles mesurant 0,2–0,4 µm. Dans les régions alvéolaires blanches, le parenchyme des rayons est complètement dégradé. Le bois sain bordant les régions alvéolaires blanches consiste habituellement en bois hâtif et en parenchyme des rayons rempli de résine. On trouve des hyphes fongiques et des trous dans le bois entourant les alvéoles, mais on n'y observe pas la pourriture typique de la carie blanche alvéolaire. En plus de cette dernière, *P. pini* cause, dans le bois voisin des sporophores et des nœuds pourris, une carie brunâtre dont les caractéristiques structurales sont semblables à celles de la carie causée par un champignon de carie blanche. On discute l'usage potentiel de la carie blanche alvéolaire comme nourriture pour le bétail et l'utilisation de *P. pini* dans les procédés biologiques de réduction en pulpe pour la production de papier.

[Traduit par le journal]

Introduction

Phellinus (Fomes) pini (Thore ex Fr.) A. Ames is one of the most destructive decay causing organisms of living trees. Throughout western United States, it is considered the single most damaging heart rot organism causing timber losses of millions of board feet each year (Scharpf 1978). The nature and extent of damage caused by *P. pini* has been reported by several investigators (Abbott 1915; Haddow 1938; Percival 1933). Growth of *P. pini* mycelia within the living tree is slow (Whitney and

Denyer 1970; Silverburg and Larsen 1967), and decay is usually considered a major problem in mature and over mature timber. However, infection can take place early causing limited damage in young trees (Skelly 1973; Haddow 1938; Boyce 1932).

The worldwide distribution of the fungus and extensive losses attributable to it, prompted Robert Hartig to investigate *P. pini* decay over 100 years ago (Hartig 1874). His excellent treatise demonstrated the nature of fungal growth in wood and gave a precursory account of the progressive stages of decay. Decay caused by *P. pini* is typically a white-pocket rot. This type of decay is characterized by spindle-shaped zones of white fibers surrounded by apparently sound wood (Figs. 1A, 1B).

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Histological studies have shown that mainly cellulosic constituents remained in white pockets (Jurasek 1964; Liese and Schmid 1966; Meier 1955). Transmission electron microscopy has provided a partial understanding of ultrastructural changes in the cell wall after decomposition by *P. pini* (Jurasek 1964; Liese and Schmid 1966; Meier 1955). However, thin sections or polystyrol replicas needed for transmission electron microscopy have limited the field of observation to changes occurring in a single tracheid. Information is not available that explains the peculiar decomposition process of *P. pini* resulting in macroscopically visible white lens-shaped pockets. This study was conducted to elucidate the decomposition process and pattern of cell wall degradation by *P. pini* using scanning electron microscopy.

Materials and methods

Three western white pine (*Pinus monticola* Dougl.) and four western larch (*Larix occidentalis* Nutt.), bearing sporophores of *Phellinus pini*, were harvested and cut into 30 cm lengths. Bolts were brought into the laboratory and aseptically split. From the exposed faces, small segments of wood were sequentially removed from the outer sapwood to inner regions of advanced decay. Radial and tangential sections were cut with a razor blade from each segment and prepared for scanning electron microscopy. Additional sections were cultured on malt agar and a selective agar medium for basidiomycetes as previously described (Blanchette and Shaw 1978). Samples of *P. pini* decayed sapwood were obtained from dead 85-year-old western larch trees. After tree mortality, decay progressed from heartwood to sapwood within the fallen tree.

Samples for scanning electron microscopy were air dried at room temperature in a dust-free environment, mounted, and coated with gold in a Techniques Hummer 5 sputtering apparatus. Fixation followed by dehydration and critical-point drying were not conducted due to the solubility of resins (that occur in decayed wood) in dehydration solutions. Specimens were examined and photographed at a 45° angle with an Etec Autoscan U-1 microscope at 20 kV.

Laboratory decay studies using western white pine sapwood blocks (2.0 cm³) were conducted using techniques previously described (Blanchette *et al.* 1978). Wood blocks were inoculated with *P. pini* (culture FP-53236-S obtained from F. F. Lombard, Forest Products Laboratory, Madison, WI) and decay chambers were maintained at 18–22°C for the duration of the study.

Results

Scanning electron microscopy revealed a distinct pattern of cell wall decomposition by *P. pini* in western white pine and western larch. During incipient stages of decay, fungal hyphae were observed in latewood (Fig. 1C), but not in earlywood tracheids of heartwood tissue. Samples of laboratory decayed white pine wood, 3 months after inoculation, revealed prolific aggregations of fungal hyphae in areas where ray parenchyma cells were in contact with tracheids (Fig. 1D). Latewood ray

parenchyma were also extensively colonized with fungal mycelia.

Severely degraded tracheids were observed in tissues from white pockets of advanced decay regions of living trees. The most striking submicroscopic characteristics of this decay were the complete degradation of middle lamellae (Fig. 1E), ray parenchyma cells (Fig. 2E), and the unusual decomposition of primary and secondary tracheid walls (Fig. 1F). The remaining cell wall structure of degraded tracheids exhibited a decay pattern different from white or brown rot decomposition as described by Cowling (1961), Blanchette *et al.* (1978), and Wilcox (1970). A substantial amount of cell wall material appeared to be left after an extensive period of decay (Fig. 1F). Degraded tracheids had a fibrillar cell wall structure (Fig. 2A). The macrofibrils (0.2–0.4 µm) (Fig. 2B) resemble the structure of cellulose lamellae described by Esau (1965). Apparently lignin had been removed from the tracheids leaving a residual cellulose framework. Tracheid walls appeared very thin in areas, and, after air drying and scanning electron microscopy preparation, tears in the wall were evident (Fig. 1F). Decomposition occurred at large distances from fungal hyphae indicating a highly diffusible lignin enzyme system was involved. Chemical analyses conducted by M. F. Effland, U.S. Forest Products Laboratory, Madison, WI, using standard techniques (Effland 1977; Moore and Johnson 1967), confirm these ultrastructural results. Decayed heartwood of western larch contained 78.16% total sugars and 16.18% lignin, whereas sound heartwood contained 69.60% total sugars and 24.06% lignin.

Typical white-pocket decay consists of white fibers surrounded by apparently sound wood. Earlywood cells were frequently found to be boundaries between white pockets and sound wood. Radial sections of white pine (Fig. 2C) and larch (Fig. 2D) indicated that decomposition of tracheids proceeded to advanced stages in latewood, but earlywood immediately adjacent was unaltered. Ray parenchyma cells were destroyed in the latewood zone. However, ray parenchyma present in earlywood tissues remained relatively unaltered. Resinous occlusions were found in sound parenchyma cells. The most severe attack of tracheids occurred in regions where ray parenchyma cells were in contact with tracheids (arrowheads, Figs. 2C, 2D). Examination of tangential sections revealed complete degradation of ray parenchyma cells (arrowheads) in tissue from white-pocket areas (Fig. 2E). Sound wood (immediately next to the white pocket)

contained nondegraded ray parenchyma filled with resinous materials (arrowheads, Fig. 2F). Although wood surrounding white pockets was not significantly degraded, fungal hyphae were present in tracheids and to a limited extent in ray parenchyma cells. As decay progressed, white pockets grew larger in latewood and coalesced in the longitudinal plane. Frequently, earlywood succumbed to the enzymatic action of the fungus resulting in white pockets extending several growth rings in width.

Although the characteristic type of decay for *P. pini* is a white-pocket rot, it is not uncommon to find degraded wood, brown in color, as well as resin-filled wood in sapwood and heartwood near sporophores or punk knots (Boyce 1961; Shigo and Marx 1977). Considerable loss of wood integrity was evident in the brownish wood, since it could easily be crushed between the fingers. Scanning electron microscopy revealed decay typical of white rot fungi rather than white-pocket rot. Erosion troughs and holes were numerous in tracheids (Fig. 3A). Selective lignin degradation was not observed. Examination of resin-filled wood revealed completely occluded tracheids (Fig. 3B).

Decomposition of larch sapwood occurring in dead *P. pini* attacked trees (decay continuing from infected heartwood after tree mortality) demonstrated a preferential attack of all latewood cells (Figs. 3C, 3D). Latewood tracheids were degraded, and cell wall decomposition was similar to that seen in Figs. 1E, 1F, 2A, 2B. Earlywood cells contained some hyphae of *P. pini*, but cell wall degradation was not extensive.

Sections from all samples of incipient and advanced decay, removed before scanning electron microscopy preparation and cultured on artificial media, yielded *P. pini*.

Discussion

Scanning electron microscopy clearly demonstrated preferential attack of lignified woody tissues by *P. pini*. General characteristics of cell wall decomposition were different from decomposition by white or brown rot fungi. White rot fungi degrade lignin and cellulose at approximately a 1:1 ratio (Cowling 1961); whereas brown rot fungi degrade cellulose leaving a relatively unaltered lignin matrix (Cowling 1961). In contrast, the white-

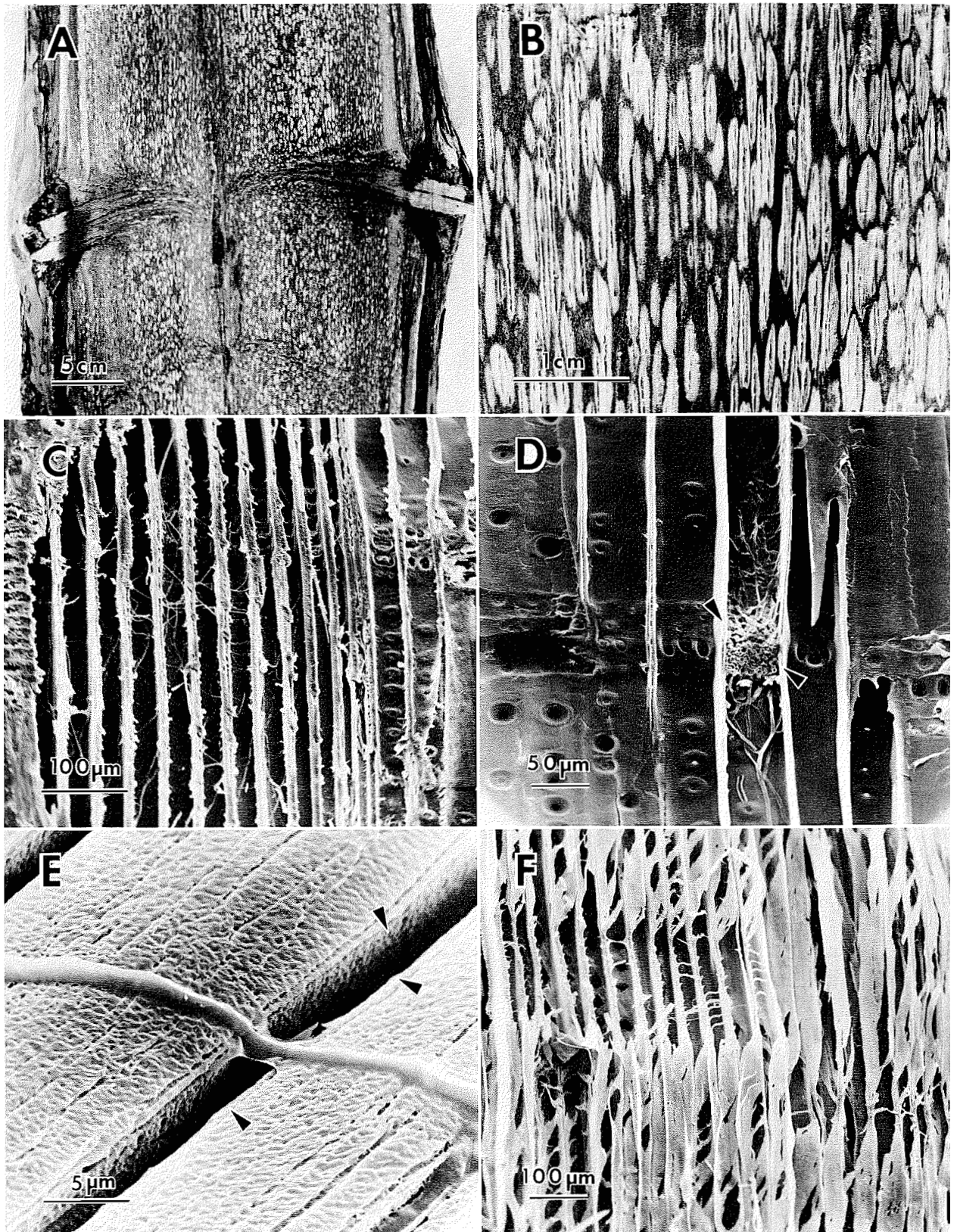
pocket rot fungus, *P. pini*, selectively attacked lignified tissue leaving a substantial amount of cellulolytic substances.

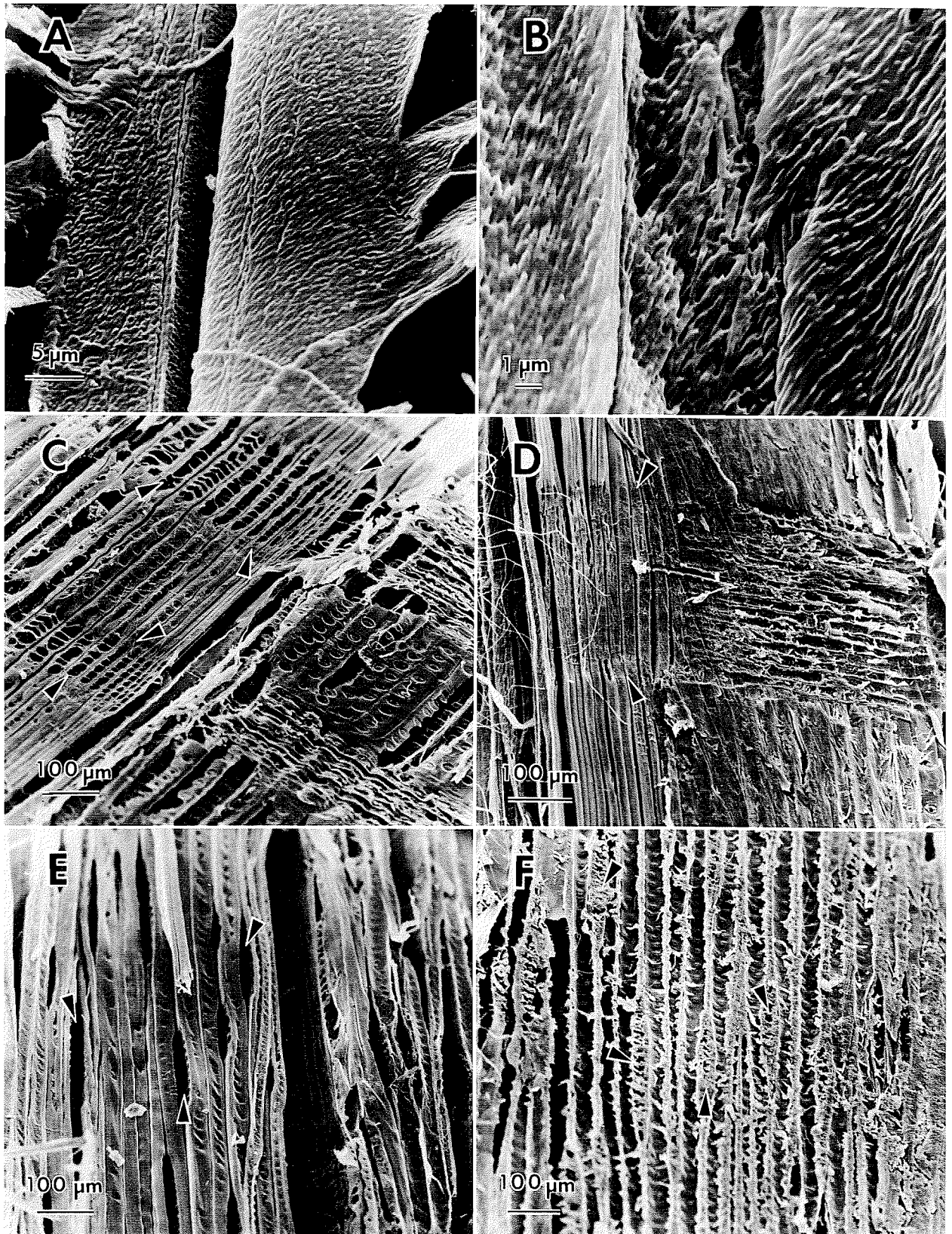
In a living tree, all tissues are not lignified to the same degree (Panshin and Zeeuw 1970). Ray parenchyma cells are more lignified than tracheids (Wilcox 1968). Within the compound woody cell wall, the middle lamella is more lignified than the primary or secondary wall. *Phellinus pini* selectively attacked latewood, degrading highly lignified tissues and cell wall components. Mycelia had been previously thought to colonize earlywood cells first rather than latewood tracheids (Boyce 1961; Scharpf 1978). This study confirms Hartig's (1874) observations that latewood tracheids are preferentially colonized and degraded. Although the reason for preferential colonization of latewood is uncertain, other fungi have also been shown to attack latewood first (Wilcox 1968).

Tracheids from within white pockets of decayed wood were severely attacked. The middle lamella was destroyed and tracheids could easily be separated from one another. The macrofibrillar structure of tracheid walls suggests lignin was removed leaving a cellulose framework. Some decomposition of polysaccharides inevitably occurs during the process of (i) complete degradation of ray parenchyma cells (Fig. 2E), (ii) thinning of tracheid walls (Figs. 1F, 2A), and (iii) extensive degradation of the tracheid wall in contact with ray parenchyma cells (Fig. 1F). No information has been reported identifying the wood carbohydrates degraded during lignin decomposition. Recent investigation with various white rot fungi (Ander and Eriksson 1977) indicates some carbohydrate loss must occur concurrently with lignin decomposition.

Chemical analyses of advanced decay confirmed the ultrastructural results presented here. A 33% loss of lignin and a 12% increase in total sugars (e.g., cellulose, hemicellulose, etc.) compared with sound wood was determined. Decayed wood used in these analyses, consisting of both white pockets and surrounding sound wood, contained less lignin and more total sugars than aspen wood (Moore and Johnson 1967). One of the limiting factors preventing the use of coniferous wood as an animal feed is the high degree of lignification. If woody tissue could be selectively delignified, the resulting wood could become an excellent livestock feed. Since *P.*

FIG. 1. (A) White-pocket rot in western larch. (B) Western white pine with white-pocket rot decay; spindle-shape zones of white fibers surrounded by apparently sound wood can be seen. (C–F) Scanning electron micrographs of incipient (C, D) and advanced decay (E, F) of white-pocket rot in white pine. (C) Fungal mycelia preferentially colonizing latewood tracheids. (D) Laboratory decayed white pine, 3 months after inoculation, showing prolific aggregations of fungal hyphae in areas where ray parenchyma cells contact tracheids (arrowheads). (E) Severely degraded tracheids with complete degradation of middle lamella (arrowheads). (F) Tracheids from white-pocket areas of decay with remaining cell wall structures.





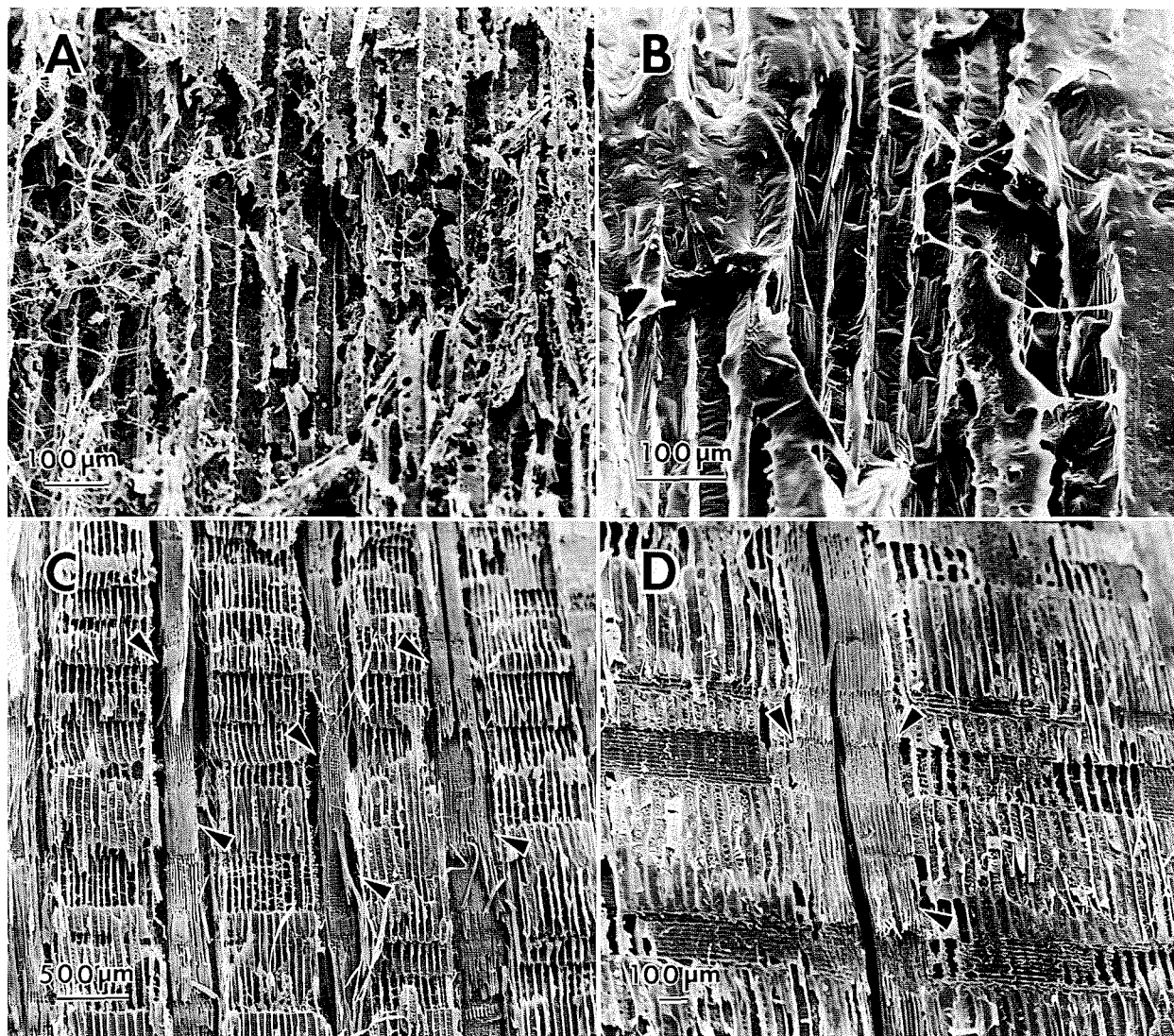


FIG. 3. (A) Decayed tracheids from brownish decayed wood in sapwood of white pine near sporophores of *P. pini*. Erosion troughs and holes in tracheids typical of white rot were present. (B) Occluded tracheids from resin-filled sapwood of white pine near sporophores of *P. pini*. (C) Degraded sapwood from dead larch sapwood showing preferential decomposition of latewood cells (arrowheads) in several annual rings. (D) Higher magnification of degraded latewood (arrowheads) with sound tissue immediately adjacent.

pini can delignify coniferous wood to levels lower than those found in aspen (a hardwood species already used as an animal feed supplement (Heaney *et al.* 1973; Mellenberger *et al.* 1971)) the resulting decayed wood could become a source of ruminant feed. Additional studies are needed to determine to what extent cellulose and other energy yielding

substances are made available and if lignin degradation by fungi results in production of growth promotants, inhibitors, or toxic substances to rumen bacterial or host animal metabolic systems.

The cause of spindle-shaped decomposition zones in white-pocket rot has been partially elucidated by this scanning electron microscopy study.

FIG. 2. (A) Macrofibrillar cell wall structure of two tracheids from white-pocket region of white pine. (B) Macrofibrils 0.2–0.4 μm in degraded cell wall. (C, D) Radial sections of white pine (C) and western larch (D) exhibiting advanced decomposition in latewood with immediately adjacent earlywood remaining unaltered. Ray parenchyma cells were completely destroyed (arrowheads) in degraded latewood. (E, F) Tangential section of white pine demonstrating decomposition of ray parenchyma in white-pocket regions (E, arrowheads) and unaltered, resin-filled ray parenchyma in adjacent sound wood (F, arrowheads).

Earlywood tracheids and resin-filled ray parenchyma are usually barriers to *P. pini* degradation. However, fungal hyphae and bore hole formation could be seen in earlywood tracheids although typical white-pocket decomposition was not observed. The resin-filled ray parenchyma of earlywood may restrict mycelial growth. In a few samples of decayed larch wood, discolored, resin-filled latewood restricted white-pocket rot decomposition. White-pocket decay occurred in several earlywood areas giving the appearance of preferential attack of earlywood as described by Boyce (1961) and Sharpf (1978). The accumulation of resinous substances in ray parenchyma of earlywood or latewood appears to be a host response to infection. Recently, the ability of oak heartwood to respond to wounding and microorganism invasion was demonstrated (Shigo and Shortle 1979). In *P. pini* attacked heartwood, ray parenchyma in some earlywood or latewood regions could have a greater capacity to respond to infection, producing more resinous substances than other areas.

Examination of decayed dead larch sapwood indicates that, without a host response, degradation proceeded to a greater extent in the latewood. The physical and chemical structure of earlywood cells (Wilson and Wellwood 1965) may be solely responsible for initial prevention of lignin degradation. As decay progresses, however, the fungus can gradually colonize earlywood tissues causing lignin degradation and coalescing of white pockets. In living trees, coalescing of white pockets encompassing several growth rings is also possible, but only in the most advanced stages of decay. If enzymatic activity of *P. pini* could be manipulated to delignify all woody tissues equally, an excellent biological pulping process for the paper making industry could be realized. Additional study of lignin degrading enzyme systems of *P. pini* as well as other white-pocket rot fungi (e.g. *Fomes nigrolimitatus* (Rom.) Egel., *Polyporus tomentosus* Fr.) should receive high research priority.

The reason *P. pini* causes a typical white rot rather than a white-pocket rot decay near sporophores in sapwood of living trees is uncertain. Although most white rot fungi impart a bleached white appearance to the substrate, several white rot fungi produce decayed wood that is brown in color (Maloy 1967; Sharpf 1978). Additional research is needed to determine the mechanisms allowing a single fungus to produce several types of decay.

The questions presented here and future work proposed demonstrate how little we understand the decay processes of one of the most common and devastating heart rot organisms. Research concerning decay microorganisms will not only pro-

vide needed basic research but is also of great potential economic importance to man.

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